DISTRIBUTION OF PAMIDRONATE BOUND TO BONE FOLLOWING LOCAL DELIVERY FROM PAMIDRONATE-PMMA BONE CEMENT

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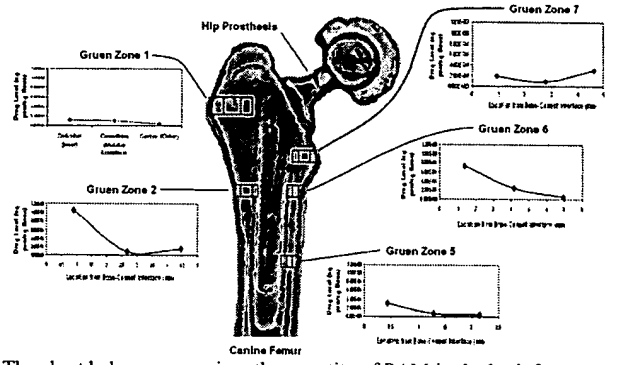
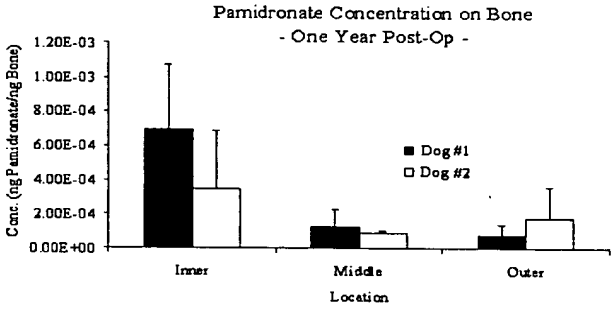
BACKGROUND: Cemented orthopaedic implants undergo time dependent aseptic loosening that is the culmination of events that begin with the formation of ultra high molecular weight polyethylene, metal, and polymethylmethacrylate (PMMA) bone cement wear debris. The debris, created by the normal stress acting on articulating components of the implant and "third body" wear, induces macrophage infiltration, a granulomatous response, and osteoclastic bone resorption. We have developed a PMMA cement formulated with the bisphosphonate pamidronate (PAM) to inhibit the osteoclast activity. The cement serves as a prosthetic grout and local depot to deliver PAM to periprosthetic bone. We tested the following hypotheses: 1) Single dose, local administration of PAM from PMMA achieves bone tissue drug levels at least as high as achieved by weekly systemic administration. 2) Physiologically active levels persist for one year in surfaces exposed to eluted drug, 3) Drug levels are inversely related to the distance of the bone from the elution fluid surface. **METHOD:** Femurs harvested from two dogs that had undergone left hip cemented arthroplasty using the PAM-PMMA cement one year prior to euthanasia were used. The canine procedures were performed under an MSKCC IACUC approved protocol. The analysis of PAM distribution within the bone required the development of an HPLC method to permit analysis of PAM in bone adjacent to PAM-PMMA cement. King & Veith's fluorescence HPLC method is the core of our method. We developed an alternative pretreatment sequence to prepare the PMMA containing bone for analysis, and also simplified their derivitazation procedure by the one step addition of o-Phthalaldehyde Reagent (OPA) for placing a fluorescent label on the PAM molecule. Current cementation of prostheses with PMMA is performed by pressure injection of the cement into the canal to ensure the interdigitation of the cement within the bone trabecula and pores. Measurement of PAM bound to periprosthetic bone adjacent to the cement requires removal of cement without causing any of the un-eluted PAM within the PMMA from binding to bone. This was accomplished by first dehydrating the femurs by soaking in 100% ethanol for 1 week and then removing the prostheses and attached cement using an oscillating saw. Sections from 5 Gruen zones that frequently exhibit debris induced osteolysis were cut and then defatted by soaking in a 2:1 methanol-chloroform solution for 2 days. Each section was further subdivided into three radial sections, i.e. inner, middle and outer by abrasion using a steel file. This resulted in 15 samples per femur; 30 samples were analyzed from both femurs. The inner sections, i.e. those in contact with the PAM-PMMA cement, underwent soaking in ethyl acetate for 2 days to dissolve the PAM-PMMA within its pores. Since PAM is insoluble in the three solvents used, the soaking sequence ensured that all PAM released from the PMMA cement remained in powder form and was removed from the bone during solvent changes. The PAM bound to bone was extracted by first pulverizing the abraded specimens into a very fine powder using a Spex Certiprep 6750 Freezer Mill and then digesting the powder using 0.2 M HCl at 50°C for 1 week (25mg bone/2ml acid). The digestate was centrifuged 10mins. at 5.2G and decanted into a Falcon tube. A 500µl aliquot of the supernatant was combined with 1 ml of 0.01M NaOH and 50µl of 10M NaOH into a 1.7ml Costar tube. This solution was mixed and then centrifuged 10mins. at 1.3g. This supernatant was discarded and the calcium salt precipitate was washed with 1 ml distilled water then centrifuged for 10mins. The precipitate was dissolved using 300µl of 0.2M H_3PO_4. Calcium was removed using 375µl of 0.2M EDTA in 0.2M NaOH and 200µl of AG 50W-X8 resin (K^+ form). This solution was vortexed and then centrifuged. Finally, a 550µl aliquot was filtered through a 0.2µm membrane, and alkalized with 10µl of 10M NaOH. From this solution 40µl was withdrawn and derivatized using 400µl of OPA. Exactly 2:00 min after the addition of OPA, 300µl was injected onto the HPLC column (Eclipse XDB-C18 5µ 25cm x 4.6mm). The mobile phase was 16% acetonitrile in a 0.025M citrate-phosphate buffer, adjusted to pH 6.5, and was pumped isocratically at 1ml/min. Detection of the PAM was performed using a fluorescence detector with excitation and emission wavelengths of 340 and 456 nm, respectively.

RESULTS: The image montage below presents the distribution profile for PAM within Dog #1's femur at 5 Gruen zones.



The chart below summarizes the quantity of PAM in the both femurs.



The table below compares PAM content in beagle sternum and iliac bone following daily oral administration over a one year period versus local administration to femur from the PAM-PMMA cement.

Mode of Pamidronate Delivery	Animal	Total Annual Drug Administration (grams)	Average Pamidronate Content (ng Pam/ng Bone)
Oral Administration			
Sternum & Iliac Bone: Year 1 Data	Beagle	91.3	3.16E-04
Local Drug Delivery via PMMA:			
Femur (all sections)	Hound	6.04E-03	2.54E-04

DISCUSSION: The percent of PAM bound to bone after 1 year for the five segments was estimated to be 96% ± 49% (mean ± std.dev.). This result was determined by first integrating the PAM concentration curves to compute the total amount of PAM within the bone segment. Next, using an ***in vitro*** elution study, we estimated the amount of PAM that leached out of the cement and contacted the bone-cement interface. That study determined that the delivery capacity of the PAM-PMMA was 173 µgrams PAM/cm^2 bone-cement contact area. The data suggest that after 12 months a significant quantity of the original PAM remains bound to bone, with the majority bound to the inner cortex or bone exposed to the effective joint space. Further, the presence of PAM on the middle and outer sections suggest that PAM was transported from the inner region. The periosteal surface at the calcar contained higher drug levels than the other regions because it was exposed to synovial fluid that recirculated PAM leached from the exposed cement at the femoral neck surface. The table compares the bone levels of PAM in our hound femurs versus published values in beagle sternum and iliac bone after one year of daily oral doses. Equivalent PAM levels were achieved from only one 15,000th of the oral administered dose. The sternum and iliac bone is more vascular than the femur, and this tissue did not undergo surgical intervention that would compromise drug delivery. Our findings support the hypotheses and highlight the superior efficiency of local drug delivery over other delivery modes.

BIBLIOGRAPHY:

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